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A disease epidemic on *Zizyphus mucronata* in the Kruger National Park caused by *Coniodictyum chevalieri*

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Abstract: This study records a severe outbreak of a disease on *Zizyphus mucronata* (*Rhamnaceae*) in Greater Kruger National Park, South Africa. The causal agent of the disease was found to be *Coniodictyum chevalieri*, a fungus previously believed to be very rare. Detailed illustrations of the symptoms and fungus are presented in order to facilitate future studies. The known geographical distribution of *Coniodictyum* is presented in relation to the distribution of its host, and a short review of its systematic history is also given. This also treats an invalidly published species name in South Africa, which has confused the literature. A DNA-based phylogeny is presented for the pathogen and this reflects the unique nature of its geographical distribution and biology.

Key words: *Basidiomycota*, *Coniodictyum*, *Cryptobasidiaceae*, epidemic fungal disease, *Exobasidiales*, Kruger National Park, South Africa, *Ustilaginomycetes*, *Zizyphus mucronata*.

INTRODUCTION

The Kruger National Park (KNP) is one of the world's most famous wildlife reserves covering an area of about 20000 km^2. It is not only home to 132 free-ranging mammals (Pienaar 1987), among them well-known mega-herbivores and large predators, but also provides the habitat for some 500 species of birds and 2000 species of higher plants; almost 400 of South Africa's approximately 1100 tree species can be found in the park (van Wyk 1984, Anderson 1999). Despite the many serious diseases that have decimated native trees in various parts of the world during the course of the last 100 years, very little is known regarding the health of native trees in southern Africa, including of the KNP. This study constitutes part of a project aimed at increasing the basic understanding of the role that diseases might play in the life cycle of a native tree species in southern Africa.

Buffalo Thorn (*Zizyphus mucronata*) is a common tree species in the Southern African Savanna and Nama Karoo biome (biome definition according to Rutherford (2003). This savanna habitat distribution is also mirrored by its overall distribution in Africa (Fig. 1). In these areas, it is found on many different soil types, but it is especially abundant on brackish flats, along rivers on alluvial soils, and it also shows a special preference for termite mounds (van Wyk 1984, Coates Palgrave 2002). Due to its abundance, *Z. mucronata* is an important food source for a great variety of animals both browsing (e.g. elephant, giraffe, black rhino, kudu) and fruit-eating (e.g. warthog, monkeys, birds). Furthermore, *Z. mucronata* plays a central role in the nutrition of a number of insects, being for example a crucial food source for the larval caterpillars of the Atlas Moths *Epiphora mythimnia* and *E. bauhiniae vera* (Pinhey 1972).

The cosmopolitan (sub-)tropically distributed genus *Zizyphus* comprises about 100, mostly very drought-tolerant species that are used by humans for many different purposes. Especially, *Z. mauritiana and Z. jujuba* have considerable economic importance as fruit trees in China and India where they had been grown for some 400 years. Various *Zizyphus* species, especially *Z. mucronata* in Africa, *Z. jujuba* in China and India, and *Z. joazeiro* in South America, are important sources of traditional African, Chinese, Indian and South American medicine and various medicinally active compounds, e.g. against fever, tumours, human parasites, have been isolated, recently (e.g. Nunes *et al*. 1987, Maurya *et al*. 1989, Arndt & Kayser 2001, Arndt *et al*. 2001).

In late March 2004, large numbers of spectacular snow-white powdery balls were observed mainly on the branches and fruits of *Z. mucronata* trees in the southern part of Greater KNP. In this study, we consider the identity of the fungus causing the epidemic gall disease on *Z. mucronata* in the KNP. The fungus is described in detail and its phylogenetic placement is determined based on DNA sequence comparisons. The associated disease is discussed and illustrated comprehensively, for the first time providing photographic records, and its relative importance is considered.

MATERIALS AND METHODS

Study area and monitoring of the disease

Greater KNP (GKNP) is situated along the north-eastern border of South Africa and extends over about 400 km from the Crocodile River in the south to the Limpopo River in the north (Figs 2, 3). It consists of the state-owned core KNP of about 20 000 km^2 and bordering private game reserves that are connected to KNP at its western border, mainly south of the Olifants River, adding approximately 2000 km^2. Details on the geology, climate and vegetation structure of KNP can be found in e.g. van Wyk (1972), and Gertenbach (1980, 1983).

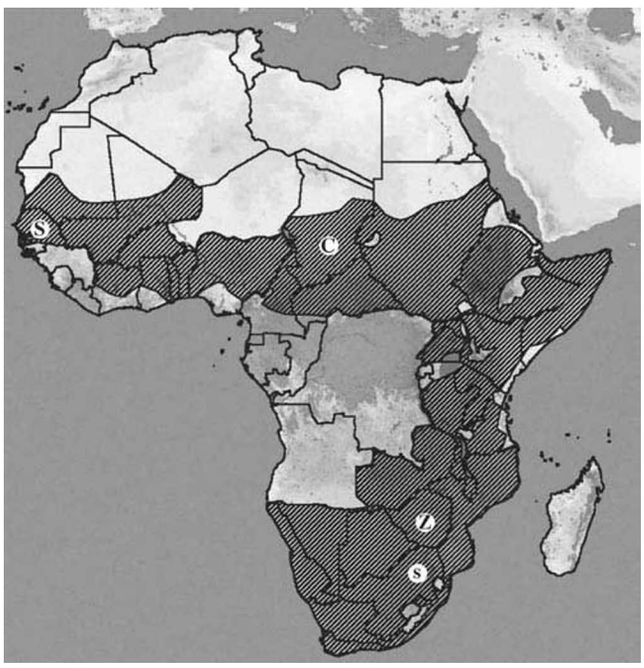

Fig. 1. Map showing the (savanna biome) distribution of *Zizyphus mucronata* in Africa (hatched). White dots represent countries in which *Coniodictyum chevalieri* has been reported so far. Characters within dots stand for: S (Senegal), C (Chad), Z (Zimbabwe), s (South Africa).

Observations for this study were made in the southern part of Greater KNP. To understand the overall distribution of the disease, we monitored the fungal galls that could be seen from the park tracks. Park rangers also monitored the distribution of the fungus with the help of GPS-devices during their routine control tours in June and July 2004. Thus, the overall distribution of the disease in the national park could be extrapolated although large parts are not easily accessible. In 2005 the same regions of the park were surveyed again for the disease. To quantify the disease prevalence at representative sites in the park, a census was conducted in Manyeleti (compare Fig. 3) in mid May 2004. Two of the census plots were approximately 2 ha in size, representing dense bushveld, a dense form of savanna, and the third was situated in a depression

Fig. 2. South Africa, its provinces, and the location of Kruger National Park.

Fig. 3. Kruger National Park and adjacent reserves (Greater KNP). The epidemic affected the whole of the southern park. Its northernmost extension was somewhere between the road Orpen-Satara and the Olifants River.

next to a dam. Each *Z. mucronata* tree in these plots was scrutinized for the conspicuous fungal galls. The presence or absence of the disease on trees was monitored at these sites.

Morphological comparisons and isolations

For light microscopy, free-hand sections of the fungal hymenium situated at the surface of the galls and detached spores were mounted either in water, clear lactophenol, cotton-blue lactic acid or Hoyer's fluid (Cunningham 1972) and examined using a Zeiss Axiovision microscope with phase contrast and interference optics. Drawings were made of both spores and the most frequent disease symptoms on branches and fruits.

Spores were also examined using scanning electron microscopy (SEM). For this purpose spores were fixed on double-sided adhesive tape on a stub and sputter-coated with gold with an E5200S sputter coater (Polaron, Watford, England). The samples were subsequently examined with a JSM-840 scanning electron microscope (JEOL, Tokyo, Japan).

To obtain cultures, spores were thinly dusted onto the surface of malt-yeast-peptone agar (Van der Walt & Yarrow 1984) in Petri dishes. The Petri dishes were kept at room temperature or in incubators at 25° C:

Cultures of the fungus have been deposited in the culture collections of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria (CMW 23046, CMW 23047).

All collections listed in Doidge (1950) under *Coniodictyum evansii* that had been deposited in the National Mycological Herbarium in Pretoria (PREM) were examined. These include herbarium accession numbers PREM 92, 1006, 1214, 2537, 5648, 8789, 10090, 11240, 11812, 15019, 20611, 30667. It was not possible to obtain the collection Rh 146, the only report on *Z. jujuba*, which had been deposited in the then Mycological Herbarium of the Department of Agriculture, Southern Rhodesia, which is now the National Herbarium of Zimbabwe. Representatives of our collections are deposited at PREM (PREM 59000-WM3450, PREM 59001-WM3488).

DNA sequence comparisons and phylogeny

In order to confirm the identity of the fungus as determined based on morphological characteristics, DNA sequence comparisons were made and phylogenetic trees were inferred. DNA was isolated directly from the spores of infected branches and fruits as well as from cultures using Qiagen Plant Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's protocols. For mechanical cell disruption, spores were crushed between microscope slides, or in the case of culture material, by using a micro pestle in an Eppendorf cup, which was cooled with liquid nitrogen. PCR and direct sequencing of both strands of the 5' end of the large subunit of the ribosomal gene cluster was performed using the primer pair LR 0R (Moncalvo *et al*. 1995) and LR 6 (Vilgalys & Hester 1990). PCR and cycle sequencing settings were the same as those described by Ritz *et al*. (2005). DNA sequencing was done on an ABI PRISM 3100™ sequencer (Perkin-Elmer, Warrington, U.K.). Contigs of the double- stranded nucleotide sequences were obtained and edited with the help of Sequencher 4.5 (Gene Codes Corporation, Ann Arbor, Michigan). All available sequences of *Cryptobasidiaceae* were obtained from GenBank and accompanied by sequences from *Graphiolaceae* and *Brachybasidiaceae*. Representatives of the latter two families were used to root the phylogenetic trees. The GenBank accession numbers follow the species names on the phylogenetic tree.

From the above sequences an alignment was produced with MAFFT 5.66 (Katoh *et al.* 2005) using the iterative refinement method with the following settings: the Needleman-Wunsch algorithm active, 2 tree rebuilding steps, 1000 iterations and default values for gap opening and gap extension penalties (NW-NS-i: −nofft −retree 2 −maxiterate 1000 [−bl 62] −op 1.530000 −ep 0.123000). Phylogenetic trees from this alignment were derived by Bio Neighbour Joining (BioNJ (Gascuel 1997) with the help of PAUP 4.0b10 (Swofford 2001) and by Bayesian inference using Metropolis Coupled Monte Carlo Markov Chains (MC^3) and MrBayes 3.1.1 (Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003), respectively. Branch support for neighbour joining was determined by 5000 bootstrap replicates. For BioNJ the best fitting model (TIMG) of DNA substitution was determined with the Akaike Information Criterion (Akaike 1974) implemented in Modeltest 3.7 (Posada & Crandall 1998) and then used to obtain both the phylogram and the bootstrap consensus tree. In the case of MC^3 the GTR+I+G model (Tavaré 1986, Yang 1993, 1994), as the most complex model, was chosen according to the simulation study results of Huelsenbeck & Rannala (2004) and default values for the *prior* settings. Three runs of MC^3 with 1.000.000 generations were performed, and every 100^{th} generation was sampled resulting in 10001 trees. The first 1001 trees were discarded and the remaining 9000 trees were used, well after the chains had converged to stationarity, to estimate the *posterior* probability distribution. One MC^3 analysis was run over 6.000.000 generations to marginalize the chance that we might have missed a higher plateau of stationarity. In this case the majority rule consensus tree was constructed from 50.000 trees and 10.001 trees were discarded as "burn-in". The sequences derived in this study have been deposited in GenBank with the following accession numbers (DQ334805, DQ334806), the alignment is lodged in TreeBase (study accession number=S1474, matrix accession number=M2652).

RESULTS

Disease symptoms

The fungus causing galls on *Z. mucronata* in this study predominantly infects branches and fruits but could also be found on the veins and peduncles of leaves. In some cases the nodes of branches were most commonly affected while in most cases the infections were randomly distributed throughout the trees. The affected organs always reacted to give rise to galls that produced an abundance of white flour-like spores. The galls ranged from few millimetres in diameter to over the size of a golf ball, seemingly correlated to the size of the plant organ affected (compare Fig. 4).

Morphological comparisons and isolations

The fungus causing the galls in this study was identified as *Coniodictyum chevalieri* Har. & Pat. This is a monotypic genus that resides in the *Cryptobasidiaceae*, *Exobasidiales*, *Ustilaginomycetes*, *Basidiomycota*. The fungus combines two very unique features that make it easily recognizable. These are the snow-white spore-producing galls breaking out of diverse organs of *Z. mucronata* and the morphology of the multi-celled spores (Figs 4 – 6). The spores are frequently composed of four longitudinally arranged sections, each of which is made up of several cells. However, there are frequently completely odd-numbered spores with extraordinary shapes (compare Fig. 6) and the general spore shape might be best characterized by " diverse and uneven". Spores were (15-)18-23(-28) µm in size. Detailed drawings of the hymenium can be found elsewhere (Malençon 1953, Oberwinkler 1977).

Using SEM, it was possible to observe for the first time that the surface of the presumed basidiospores is covered more or less densely with small warts (Fig. 6) that cannot be seen with the light microscope. The spores germinated on artificial media, at first producing conidia and yeast cells (for detailed microscopical descriptions of these compare Malençon (1953). Cultures grew very slowly (*ca*. 1 mm diam after 5 – 7 d). After about 3 – 5 wk, the single-spore cultures had transformed into a solid slightly salmon-coloured compact hyphal mass displaying a brain-like surface structure.

The collections of *C*. *chevalieri* deposited in PREM had been collected in the following provinces of South

Fig. 4. Disease symptoms associated with *C*. *chevalieri* infection. A. Young infections erupting mainly from the nodes of a branch causing wilting of apical leaves. B. Fully developed infections on a branch from which most leaves were lost. C. Golf ball-sized gall strangling a branch. D. Almost all fruits had been infected on some trees. E. Young fruit infection showing seven galls just erupting from a single fruit. F. Infection at the peak where the fruit was completely transformed into several galls. G. Severe infections leading to branch death.

Africa: Limpopo, Mpumalanga, KwaZulu-Natal and Gauteng. They include the type collection of *Hyalodema evansii* (PREM 92). Nine of the twelve specimens had initially been labelled as *H. evansii* P. Magnus before being transferred to *C. chevalieri* Har. & Pat. The remaining three specimens (PREM 92, 2537, 5648) had been labelled as *Coniodictyum evansii* P. Magn. This is also the name that was used in Doidge's compendium on the Southern African fungi (Doidge 1950). However, this is not a valid name as discussed below. Doidge (1950) also lists a collection from Zimbabwe that was reported from *Z. jujuba*.

Distribution and prevalence of the disease in 2004 and 2005

The disease was first discovered in the southern parts of the park in late March 2004, in the area of the camps Skukuza, Orpen, and Lower Sabie. The peak of the disease was reached in May/June when it was detected to have spread over a distance of about 200 km on the north-south axis and the entire east-west extension of the park (Fig. 3). Infections remained clearly visible on trees until August. The census taken at the two plots representing dense bushveld revealed that all 43 and 53 trees, respectively, in these plots were diseased. At the third plot next to the dam, all 38 trees counted were diseased. This amounted to a disease prevalence of 100 % in the region. The majority of trees at all three plots were heavily infected, however medium-infected trees and trees with hardly any infection could also be found.

During the first half of 2005, rangers did not notice signs of the disease in the park. Likewise, symptoms were not observed in roadside surveys of *Z. mucronata* trees undertaken during April and June 2005. The three reference plots were, therefore, closely investigated on foot. None of the trees in the two bushveld reference plots that had displayed 100 % disease incidence in the previous year showed fresh infections. However, viable spores that could

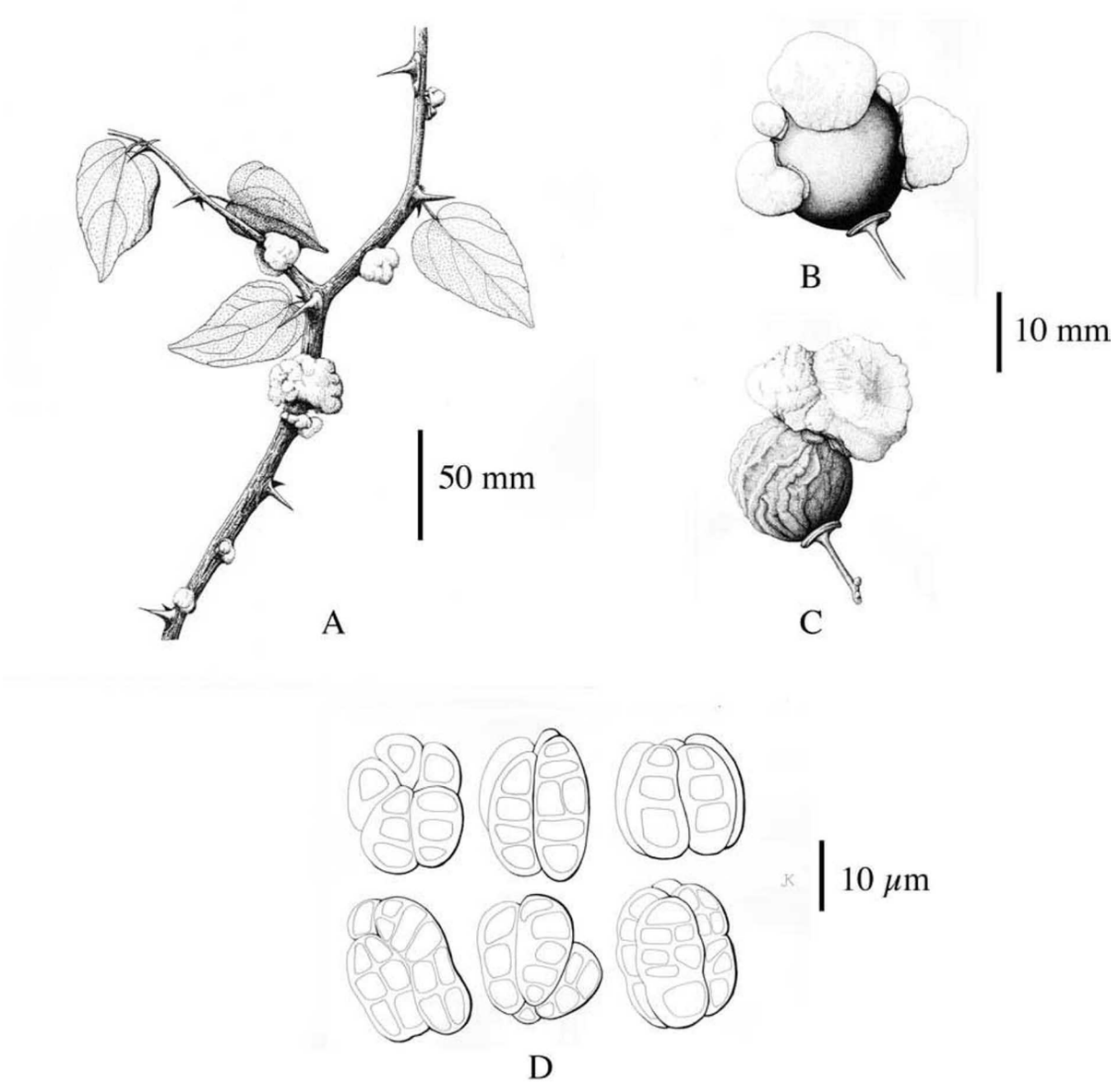

Fig. 5. Line drawings of disease symptoms associated with *C. chevalieri* infection. A. Symptoms caused by *C. chevalieri* on a branch of *Z. mucronata.* B. Fruit infection at the peak of its development. C. Later-stage fruit infection causing the fruit to shrink and wrinkle. D. Basidiospores of *C. chevalieri*.

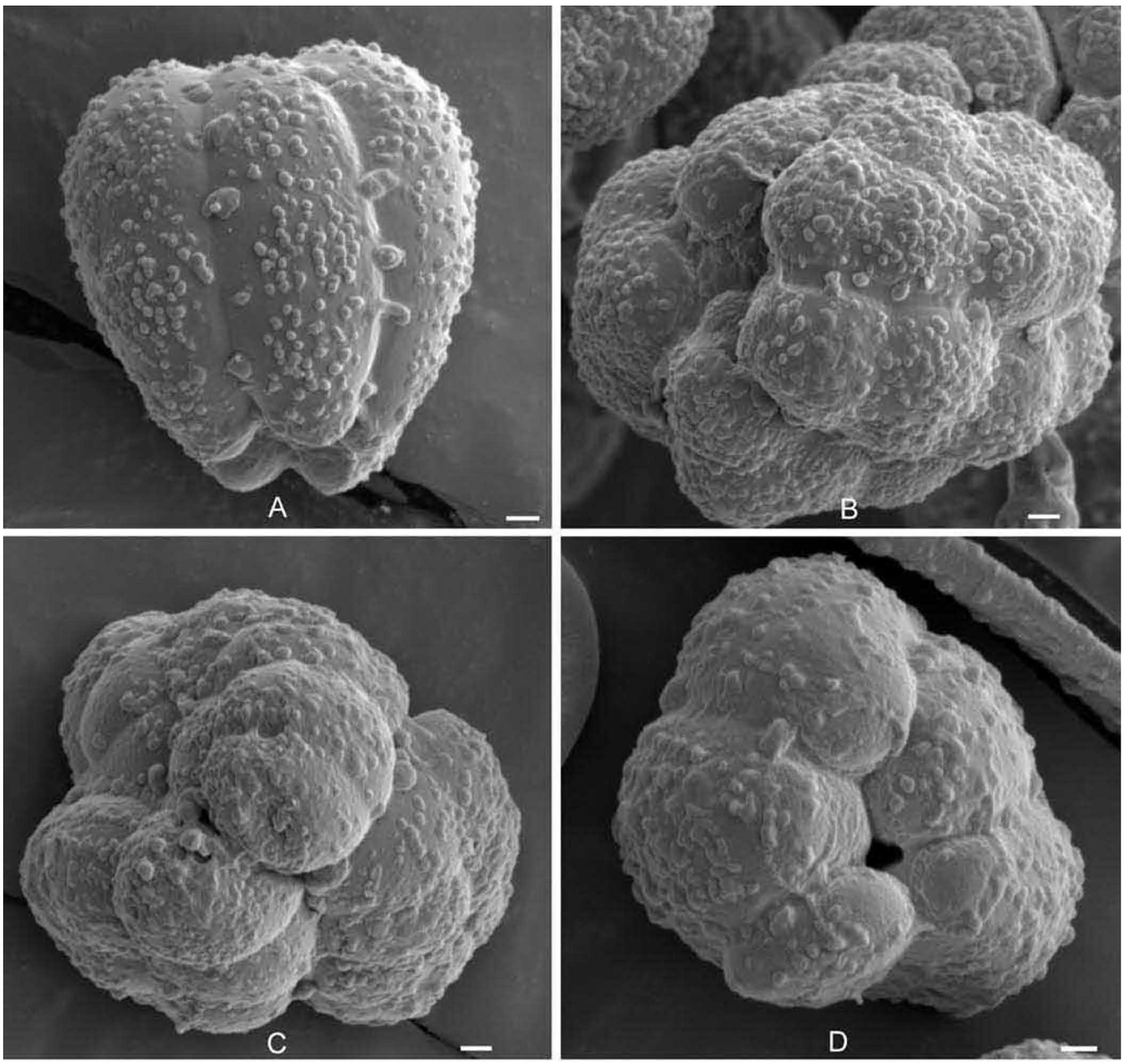

Fig. 6. SEM pictures of *C. chevalieri* basidiospores. A–B. Uniformly shaped spores with four longitudinal sections, each comprising several cells. Warts on spore surface. C. Completely irregular spore form. D. Spore consisting of five longitudinal units. Scale bars = 1 µm.

be germinated on MYP agar were obtained from two galls from previous year infections. All of the trees were alive but many of the heavily infected branches, easily detectable by the presence of old galls, had died. There was also practically no fruit production in 2005, while the trees had produced abundant fruit at the same time in the previous year. The reference trees close to the dam had recovered more effectively than the trees at the two other census sites. They also displayed abundant fruit production, and small numbers of weak infections could be found on the fruits of six trees.

DNA sequence comparisons and phylogeny

Sequences were obtained from spores of different host organs (branches, fruits) of different trees from different sample sites as well as from cultures grown from spores. The sequences spanned the D1 – D3 region of the nuc LSU rDNA with a length of about 1000 bp. All eight sequences obtained were identical. They were also identical to the only available sequence of *C. chevalierin* in GenBank that had been deposited for a study of the *Exobasidiales* (Begerow *et al*. 2002). This sequence was derived from a culture obtained from material collected by Johannes van der Walt in 1990 around Skukuza camp, also in KNP.

The final alignment used for the phylogenetic analyses was restricted to the D1/D2 region, due to the length of the sequences deposited in Genbank, and comprised 508 base pairs. Tree topologies obtained by four different runs of MC^3 were identical. Tree topologies obtained by MC^3 versus BioNJ were almost identical. The only difference was that the four *Laurobasidium* specimens were resolved as a monophyletic group in MC^3, whereas in BioNJ *Laurobasidium lauri* was the

sister group to the three samples of *Laurobasidium hachijoense* together with the *Clinoconidium* spp. as a whole. The *posterior* probabilities and bootstrap values were similar (compare Fig. 7). The most important support values for this study are those for *Cryptobasidiaceae* (1.00 posterior probability / 99 % bootstrap) and those for the split between *Coniodictyum* (1.0/100) and the rest of the *Cryptobasidiaceae* (1.00/100).

Both *Clinoconidium* and *Laurobasidium* were resolved as monophyletic by MC^3, but with low support values, while in BioNJ only *Clinoconidium* was monophyletic, however, also just weakly supported by bootstrap support. Some intraspecific structure was observed within *Clinoconidium bullatum* and *C*. cf. *bullatum*, respectively. The monophyly of the outgroup genera *Kordyana* (1.0/100) and *Graphiola* (0.96/100) was highly supported. However, *Dicellomyces* did not form a monophyletic group with *Kordyana*, which resides in the same family, the *Brachybasidiaceae*, but with *Graphiola*, which resides in the *Graphiolaceae*.

DISCUSSION

Although it has long been known as geographically wide-spread in Africa, *C. chevalieri* is an unusual fungus with poorly known ecology and infection biology. This study emerged from an unusually severe outbreak of the pathogen in an ecologically important and sensitive

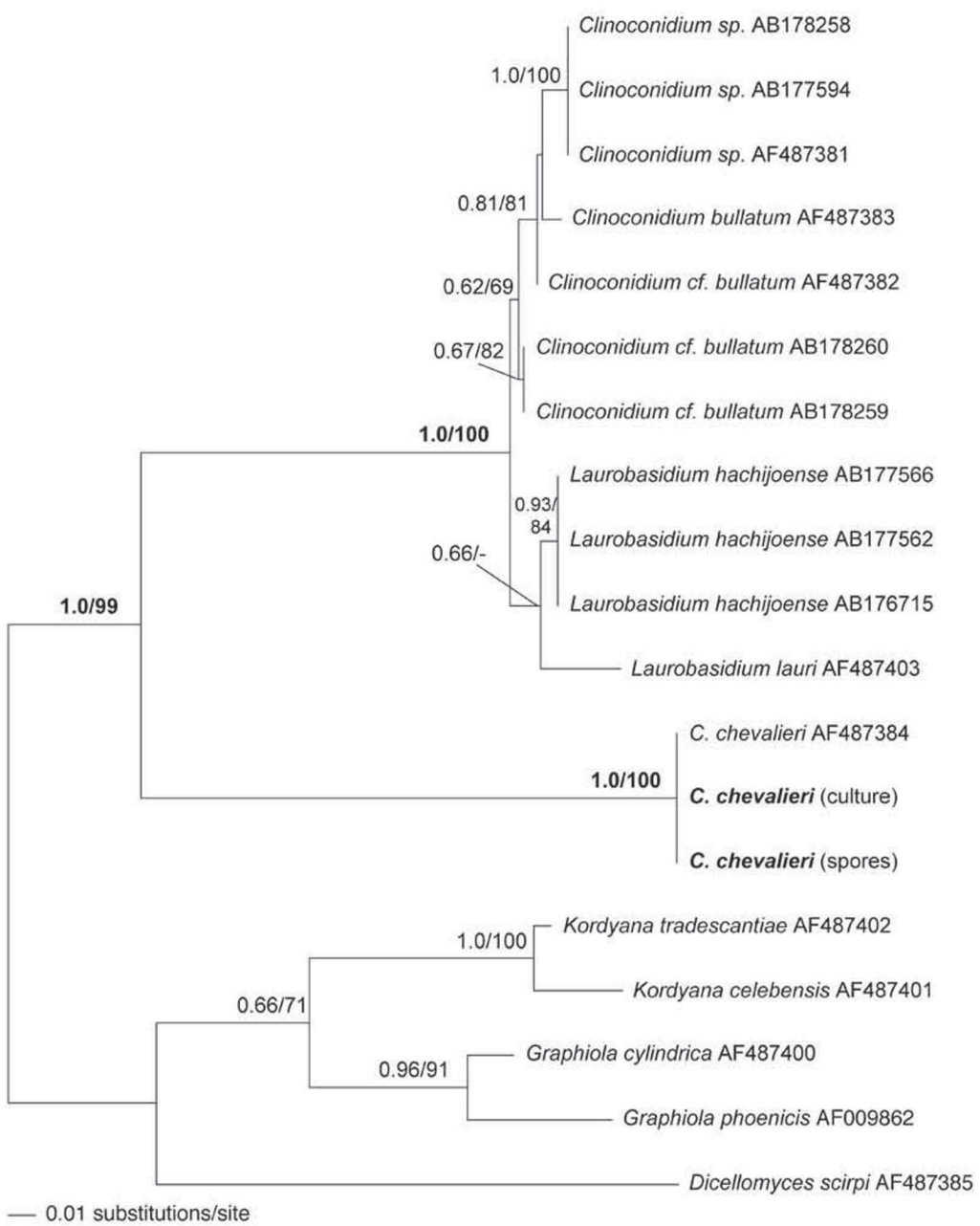

Fig. 7. Phylogeny of *Cryptobasidiaceae* showing the position of *Coniodictyum chevalieri* based on Bayesian MC^3 with GTRI+G as base substitution model. The depicted phylogram was calculated from 9000 trees after the Markov chains had converged. MCMC (as fractions of one) and bootstrap support values (derived by BioNJ with TIM+G as nucleotide substitution model) are given above branches. Only values greater than 60 % are shown.

part of South Africa, where the disease raised concern amongst rangers and naturalists. It provided an opportunity to critically review what has been known about it, to clarify some misconceptions of earlier studies, and it thus provides a foundation for future studies. *Coniodictyum* has had a complicated systematic history. It has had three different names assigned to it and it has also resided in the Ascomycota for a period of time. Malençon (1953), in his exceptionally thorough study on the life cycle and systematic relationships of the fungus, gave also a first account of the systematic history of *C*. *chevalieri*. However, he was not aware how frequently the fungus had been collected in South Africa. This led to incorrect conclusions regarding its levels of occurrence.

Coniodictyum chevalieri was first described in 1909 from Chad on *Zizyphus baclei* = *Z*. *mucronata* (Hariot & Patouillard 1909) where it had been collected on fruits in March and November 1903 by Chevalier. In 1910, Magnus (1910) described *Hyalodema evansii* based on material collected by Pole Evans in 1906 at Zoutpansberg (Limpopo Province), South Africa, on *Zizyphus* sp., which was later also identified as *Z*. *mucronata* (Malençon 1953). Doidge (1950) cited the fungus under *Coniodictyum evansii* (Magnus) Höhn. without providing justification for this new combination. This is especially important because von Höhnel (1910, 1911) himself argued strongly for the conspecificity of *C*. *chevalieri* and *H*. *evansii* with *C*. *chevalieri* having priority, and therefore never made a new combination for this fungus. Thus, the only evident explanation for this inconsistency is that an error was inadvertently made with the epithet of Magnus' invalid description being mistakenly attached to the valid older genus name. *Coniodictyum evansii* (Doidge 1950) is, therefore, a synonymous *nomen nudum* for *C*. *chevalieri*.

The affinities of the fungus now known as *C*. *chevalieri* were first believed to be with the ascomycetes, and it was relegated to either the *Hyphomycetes Mucedineae* (Hariot & Patouillard 1909), the *Melanconieae* (von Höhnel 1911, Maublanc 1914) or the *Mucedinaceae*, *Moniliales* (Doidge 1950). However, already von Höhnel (1911) noted that some features of the hypertrophic growth resembled that of *Exobasidium*. When Malençon (1953) received abundant fresh material of the fungus collected by Th. Monod close to Dakar, Senegal, he performed an extensive morphological study concluding that the "conidiophores" producing the abundance of white spores were in reality basidia, and the spores hence basidiospores. He also, again, connected *Coniodictyum* systematically with gall-producing fungi described from *Lauraceae* of Central and South America in the genera *Botryoconis* and *Clinoconidium* as Maublanc had proposed before him, but then still under the ascomycetous *Melanconieae* (Maublanc 1914). Thus, after he had re-examined *Botryoconis*, *Clinoconidium* and *Drepanoconis*, Malençon named the family *Cryptobasidieae* (now *Cryptobasidiaceae* (Donk 1956) in honour of Alfred Lendner. This mycologist was the first to realise the basidiomycetous affinities of one of its members and had introduced the name *Cryptobasidium* (Lendner 1920), which was reduced to synonymy with *Botryoconis*. H. Sydow, like Maublanc, originally retained *Botryoconis* in the *Melanconiaceae*, but was convinced by Lendner's interpretations, concluding "Ich glaube nun, daß *Clinoconidium* und *Botryoconis* Basidiomyceten sind" and therefore transferred them accordingly (Sydow 1925). Only recently was *Laurobasidium* transferred from the *Exobasidiaceae* to the *Cryptobasidiaceae* (Begerow *et al*. 2002). Therefore, the *Cryptobasidiaceae* currently comprise five genera and seven species (compare Hendrichs *et al.* 2003).

The *Cryptobasidiaceae* have recently been confirmed to be monophyletic by Begerow *et al*. (2002), but the statistical support for the group in that study was low (obtaining a maximum of 59 % bootstrap). Our analyses, however, show that the family is highly supported both by bootstrap and Bayesian posterior probabilities. This result has obviously arisen from the larger taxon sampling within the *Cryptobasidiaceae*, while using the same gene region. This was especially possible, because additional sequences of *Botryoconis* and *Laurobasidium* had been deposited to GenBank by Nagao, Sato and Kakishima in 2004.

The only representative of the *Cryptobasidiaceae* in Africa, *C*. *chevalieri*, is also unique in its host preference and in its ecological occurrence in arid savanna biomes. This is markedly different to other members of the *Cryptobasidiaceae* that inhabit moist sub-tropical and tropical forests outside Africa attacking various genera in the laurel family while *C*. *chevalieri* so far has only been reported with certainty from *Z*. *mucronata*, a member of the *Rhamnaceae*. This unique biology, regarding its biogeography, ecology and host specificity, is reflected by the phylogenetic position of *Coniodictyum*, which is a sister taxon separated from the other members of the family that parasitize Lauraceae, by a long genetic distance and perfect support values (Fig. 7).

The potential of *C*. *chevalieri* to infect other members of the genus *Zizyphus* should be considered. The report in Doidge (1950) of *C*. *chevalieri* infecting *Z. jujuba (Z. mauritiana*?) is interesting in terms of the capacity of the pathogen to move to new hosts. However, the validity of the report could not be tested in this study and is regarded as rather doubtful. If the report were correct, it would have serious implications for countries like China and India where *Z. mauritania and Z. jujuba* are extensively grown for fruit production.

Malençon (1953) was convinced that *Coniodictyum* is a rare fungus ("en réalité est un champignon peu commun"). This is because he knew of no additional collections subsequent to the first collections from Chad and South Africa in 1903 and 1906, respectively, and the material that was sent to him from Senegal almost 50 years later. However, twelve collections made in South Africa over a considerable geographic range (compare above) and one in Zimbabwe between 1910 and 1938 documented in Doidge (1950) clearly escaped Malençon´s notice. Thus, his statement regarding the rarity of the fungus is based on the incorrect assumption that the fungus had been collected

only twice before he received the material from Dakar. Furthermore, the fungus was also collected in more recent years in KNP by Johannes van der Walt in 1974 and again in 1990 close to the camp-sites “Skukuza” and “Lower Sabie”, respectively. It is however important to note that the fungus was almost absent from KNP in 2005, thus showing great fluctuations in its prevalence in different years. At this stage we speculate that the extensive spread of the fungus in 2004 was boosted by much higher rainfalls between January and April 2004, compared to the same months in 2005 (data not shown). Nevertheless, long-term observations are needed to either prove or disprove this hypothesis.

Another reason why *C. chevalieri* might not be as rare as previously believed, is provided by old galls found on branches of *Z. mucronata*. These indicate that the fungus had been present in Kruger Park in recent years. The frequency of collections of *C. chevalieri*, is, probably mainly determined by the number and activity of mycologists in areas of Africa, where *Z. mucronata* grows and we assume that it most likely could be found in the whole range of its host’s distribution if extensively looked for. The situation appears to be similar in the representatives of *Cryptobasidiaceae* in tropical America where specimens have been recollected in Costa Rica in the late 1990s after a period of about 60 years absence of reports of these fungi (Gómez *et al.* 1998).

In 2004, many *Z. mucronata* trees were so heavily stressed by the production of large galls that we predicted large-scale death during the dry winter months. However, almost all infected trees remained alive in 2005 and appeared to have recovered well. This rapid recovery of *Z. mucronata* from the severe infection by C. *chevalieri* in 2004 is consistent with observations of rapid recovery and vigorous resprouting of Buffalo Thorn after fire damage. However, hardly any fruit could be found on the trees the year after they had been heavily infected at the two bushveld plots. We speculate that stress due to infection by C. *chevalieri* reduced plant vigour and consequently flower and fruit production in 2005. The fact that trees close to the dam had recovered well and produced abundant fruit, despite their being heavily infected in 2004, is probably due to favourable edaphic conditions at this site, with higher water availability, which reduced the impact of stress due to the disease.

This study represents the first report of an epidemic caused by *C. chevalieri*, a fungus previously believed to be extremely rare. Contrary to views regarding its rarity, we were able to show that *C. chevalieri* has been collected regularly, especially between 1906 and 1938, in various parts of South Africa. The infection status and the health of the trees in reference plots in KNP is being monitored and it is hoped that during coming years new knowledge concerning the ecology of the pathogen and the conditions favouring its spread will emerge. These will be potentially useful in developing hypotheses regarding modes of distribution and ecological factors that might have an effect on the survival and spread of the fungus.

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